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Abstract

Background: Chronic diseases, including coronary heart disease, have been associated with ingestion of drinking water with high levels of inorganic arsenic (over 1000 μg/L). However, associations have been inconclusive in populations with lower levels (<100 μg/L) of inorganic arsenic exposure.

Objectives: We conducted a case-cohort study based on individual estimates of lifetime arsenic exposure to examine the relationship between chronic low-level arsenic exposure and risk of CHD.

Methods: This study included 555 participants with 96 CHD events diagnosed between 1984 and 1998 for which individual lifetime arsenic exposure estimates were determined using data from structured interviews and secondary data sources to determine lifetime residence which was linked to a, geospatial model of arsenic concentrations in drinking water, which were correlated with historically collected urinary arsenic concentrations. A Cox proportional hazards model with time-dependent CHD risk factors was used to assess the association between lifetime exposure to low-level inorganic arsenic in drinking water and incident CHD.

Results: We estimated a positive association between low-level inorganic arsenic exposure and CHD risk (Hazard Ratio (HR): =1.38, 95%=1.09 1.78 per 15 μg/L) while adjusting for age, gender, first-degree family history of CHD, and serum low density lipoprotein levels. The risk of CHD increased monotonically with increasing TWAs for inorganic arsenic exposure in water relative to < 20 μg/L (HR=1.2; 95% CI: 0.6, 2.2 for 20-30 μg/L, HR=2.2; 95% CI: 1.2, 4.0 for 30-45 μg/L, and HR=3; 95% CI: 1.1, 9.1 for 45-88 μg/L).

Conclusions: Lifetime exposure to low-level inorganic arsenic in drinking water was associated with increased risk for CHD in this population.

Introduction

Non-occupational exposure to inorganic arsenic occurs mainly through drinking contaminated water (EPA, 1988). In recent decades, research has identified a relationship between exposure to high concentrations of inorganic arsenic in drinking water and the risk of coronary heart disease (CHD); however the risk at lower levels is ambiguous. Studies from Asia, where water concentrations of inorganic arsenic can be over 1000 μg/L, have reported inorganic arsenic in drinking water to be associated with ischemic heart disease and carotid atherosclerosis (Chen et al. 1996; Tseng et al. 2004; Wang et al. 2002), hypertension (Chen et al. 1995, 2007; Chen et al. 2006b; Rahman et al. 1999), and intermediate outcomes associated with CHD including, carotid artery intimal-medial thickness (Chen et al. 2006a) and ECG changes (Wang et al. 2010).

An association of cardiovascular risk with low-level arsenic exposure in drinking water (<100 μg/L) has been suggested by recent studies (Chen et al. 2011 and Moon et al. 2013). In a study by Chen et al. 2011, results suggested a higher cardiovascular mortality rate with exposure to drinking water arsenic concentrations > 12 μg/L, and an increasing trend in hazard ratios with increasing arsenic exposure (log rank trend test=0.0019) while controlling for known CHD risk factors. Positive associations were reported by other studies with similar exposure levels (< 100 μg/L) (Medrano et al. 2010; Sohel et al. 2009). These findings suggest that increased risk for cardiovascular disease occur at levels similar to concentrations found in drinking water in areas of the United States (US).

In the US, where arsenic concentrations are generally less than 100 μg/L, ecologic studies (Engel and Smith 1994; Engel et al. 1994; Lewis et al. 1999; Meliker et al. 2007; Zierold et al. 2004) and review articles (Navas-Acien et al. 2005; Wang et al. 2007) have suggested a possible

association of drinking water arsenic with CHD, hypertension, and carotid intimal thickness. However it has only been recently that chronic exposure to low to moderate level inorganic arsenic in drinking water has been investigated as an independent risk factor for cardiovascular diseases in a prospective study. Moon et al. 2013 reported an association between urinary arsenic concentrations and coronary heart disease (HR=1.16; 95% CI: 1.03, 1.30 per 9.9 μg/g adjusted for CHD risk factors) in US American Indian communities. These findings in the Strong Heart Study were the first to prospectively assess low to moderate level inorganic arsenic exposure in urine with cardiovascular disease at a community level; however, the study was limited in assessing exposure at the individual level. Future research that prospectively follows a cohort representative of US communities with individual level exposure assessment is necessary to further substantiate the association between inorganic arsenic exposure in drinking water and cardiovascular disease and elucidate the dose response curve.

Methods

We investigated the relationship between lifetime inorganic arsenic exposures and the risk of incident CHD using a case-cohort design within the San Luis Valley Diabetes Study (SLVDS). SLVDS is a population-based prospective study conducted from 1984 to 1998 in Alamosa and Conejos counties of south central Colorado investigating the risk factors for diabetes mellitus and other related chronic diseases in Hispanic and non-Hispanic whites aged 20-74 years old. SLVDS data collection methods and participant recruitment have been described elsewhere (Hamman et al. 1989). In brief, researchers collected clinical, behavioral, and demographic data and diagnostic assessments including diagnoses of CHD from 1984 to 1988 (Hamman et al. 1989). Participants were then invited to attend follow up visits every four years through 1998 to update their behavioral, demographic, clinical assessments, and an additional set of assessments

on participants with impaired glucose tolerance at the initial visit. All participants were followed between clinic visits with telephone interviews and searches of vital statistics records to track vital status and identify underlying cause of death, where applicable (Hokanson et al. 2002). This cohort is stable with a 98 percent follow up of study participants through 1998 (Hokanson et al. 2002).

There were 1297 SLVDS participants with no known CHD events or a diagnosis of DM prior to the baseline visit. Participants with a documented refusal for re-contact in SLVDS (n=361) were excluded leaving 936 participants eligible for this study. Cases of CHD included all eligible participants with a documented CHD event between their baseline visit and 1998. A CHD event was defined as any of the following: myocardial infarction, angioplasty, and death due to acute, subacute, or chronic ischemic heart disease (codes 410-414). Potential CHD events were identified through self-report on yearly follow-up phone calls, obituary monitoring, and death certificate searches (Swenson et al. 2001). The medical records of identified CHD events were reviewed by a three-member committee of medical physicians for case confirmation.

The subcohort for estimating person years of risk was randomly selected from the eligible participants without a previous diagnosis of CHD at the time of initial enrollment. The sample size of the subcohort was determined in Pass® software based on recent research to estimate effect size (a relative risk of 1.4 for an increased risk greater than 10μg/L) (Zierold et al. 2004) with alpha=0.05 and power of 80 percent. The subcohort included 533 randomly selected participants of which 74 were incident CHD cases. The remaining 22 incident CHD cases not selected were added to the subcohort for a total of 555 participants in the CHD case-cohort study. Within the 555 study subjects, we had 64% (n=357) participation rate, 33% (n=189 (30% non-cases and 46% cases) unable to locate, and 3% (n=19) who refused participation.

Estimating arsenic exposures

Residential history (determined from structured interviews or secondary data sources) was linked to a geospatial model of predicted inorganic arsenic in groundwater to reconstruct annual estimated exposure to inorganic arsenic in drinking water over each participant's lifetime. The use of inorganic arsenic levels in residential drinking water to assess exposure was supported by findings from our research that correlated annual predicted arsenic exposure estimates with temporally concurrent speciated inorganic arsenic concentrations in historically collected samples. Between 2006-2008, study subjects or next of kin of deceased subjects as designated in SLVDS (14.2%) were contacted by mail with information about the study, followed by a call to set up an appointment for an interview and water sample collection. During the interviews (n=357, 64%) we collected data about past residences, past workplace/school locations, and history of drinking water consumption at each location. For each location, data included addresses, residence dates, water source (well or public), water treatment device (if yes, type, model number), number of glasses of water consumed per day (non-bottled water), number of glasses of beverages made with water (coffee, tea, juice, etc.), whether they typically cooked with water from the tap in the home, and whether they had a vegetable garden (if yes, which vegetables). In subjects or next of kin who were not able to be located for an interview (n=189, 33%), we utilized triangulation methods incorporating records from the county assessor's office and SLVDS tracking database to reconstruct residential history. In brief, 189 participants were not interviewed (n= 2023 person years) therefore the SLVDS contact tracking database was used to determine residence history back to 1975 and earlier for participants who reported living at the residence listed in 1975, prior to 1975. This left 914 person years across 98 subjects still missing residential history and therefore were further investigated in County Clerk records. In the end,

there were 18 subjects with partial residential history (prior to 1975) (n= 126 person years) with missing residential locations and these were assigned the mean value for the last known city of residence.

Drinking water samples were collected from the residential kitchen tap at time of interview and analyzed by the chemistry laboratory of the Colorado Department of Public Health and Environment using standard Ion Chromatography (IC) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with a detection limit of 1 μg/L. Samples with arsenic concentrations below the detection limit were given a value of half of the detection limit similar to other studies (Ayotte et al, 2006). Samples collected from private wells were assigned geographic coordinates using a global positioning system (GPS) unit (n=248). In other work we detail our methods for determining and validating the temporal and spatial variability of inorganic arsenic in groundwater in the SLV (James et al. 2013). In brief, findings indicate that naturally occurring inorganic arsenic concentrations in groundwater are stable over decades, (consistent with other research (Steinmaus, 2005)) justifying the use of geospatial models based on the mean arsenic concentration in individual private wells over decades to predict spatial variability of inorganic arsenic in groundwater.) To predict spatial variability of inorganic arsenic in groundwater which was supported by a correlation analysis in a 10 percent sample of observed and predicted values (ρ=0.715; 95% CI=0.67, 0.75) (James et al., 2014).

An exposure matrix was developed to estimate each participant's annual exposure to arsenic in drinking water. Each record included residential, employment, and school location and an estimate of the amount of water ingested and water arsenic concentration (either observed or predicted) for each participant for each year of life from birth to death or 1998 whichever came first. Three estimated exposure values were calculated for each year of the follow-up period 1984

through 1998 or CHD diagnosis which ever came first. The three estimated exposure values residential arsenic concentration (arsenic concentration in drinking water at residence), residential arsenic dose (residential arsenic concentration in drinking water times the amount of water consumed in liters per day), and total arsenic dose (residential arsenic dose plus workplace and/or school dose) respectively were each defined based on a time-weighted average (TWA). A time-weighted average (TWA) for each exposure metric was calculated by dividing cumulative per-person arsenic exposure by the number of years in each participant's lifetime to get an annuitized exposure per year (Meliker et al. 2010).

To determine which TWA exposure estimate best approximated biologic exposure, we correlated these with speciated arsenic concentrations in historically collected urine samples (collected 1984-1991), adjusting for gender and creatinine (James et al. 2013). In brief, estimates of residential arsenic concentration (R^2=0.37; ρ=0.61) were the strongest correlates of the sum of the toxic urine arsenic species (As^{3+}, As^{5+}, dimethylarsinic acid, monomethylarsinic acid), as opposed to estimates that included water consumption (residential dose) (R^2=0.21; ρ=0.46) or additionally, exposure at work or school (total dose) (R^2=0.23; ρ=0.48).

Statistical analyses

We utilized a Cox proportional hazards model incorporating a robust variance estimator specific for case-cohort study designs (Barlow et al. 1999) to examine the association between TWA inorganic arsenic exposure and diagnosis of or death from CHD. We scaled the continuous arsenic exposure estimate to the inter-quartile range (IQR) (15 μg/L), along with other continuous covariates, similar to methods used in Lin and Huang 1995.

As described above, participants had longitudinal data from two to four study visits including information on known risk factors for CHD. Risk factors for CHD believed to be independent of the mechanistic pathways proposed for arsenic were included in the proportional hazards multivariate model as time-dependent covariates (lipid measurements, BMI, physical activity, smoking, alcohol and water consumption). The univariate model included TWA inorganic arsenic as a continuous value scaled to the interquartile range (15µg/L). Person-years and exposure were censored for CHD cases at time of diagnosis. The full model included demographic risk factors ethnicity (White non-Hispanic: Hispanic), gender (male: female), and annual household income (high >=$20,000: low < $20,000); known risk factors first degree family history of CHD (no: yes), body mass index (BMI: interquartile range scaled, median=26.7, IQR=23.8, 29.3), diabetes diagnosis prior to CHD (no: yes); behavioral risk factors including current smoking status (no: yes), alcohol consumption (low <=168 grams/week: high > 168 grams/week), and physical activity level (active: sedentary) (Mayer, 1991); and continuous clinical risk factors including serum lipid measurements (high-density lipoprotein (HDL), triglycerides, and low density lipoprotein (LDL) in milligrams per deciliter (mg/dl)), hypertension (blood pressure > 140/90 or on anti-hypertensive medicine) and folate and selenium intake (micrograms). Triglyceride and HDL levels were determined using enzymatic methods and LDL levels were calculated using the Friedewald equation (Friedewald et al. 1972) and folate and selenium intake were estimated base on 24-hour diet recall involving two and three dimension visual aids for portion approximation. Nutritional analysis was based on version 14 of the Nutrition Coordinating Center's nutrient database released in 1987. Vitamin supplement use was assessed through self-report using vitamin bottle labels

In addition a final parsimonious model incorporating statistically significant covariates to the model based on a ten percent change to the HR for TWA arsenic exposure. Known independent risk factors, including gender and family history, that were significantly associated with the outcome were maintained in all models regardless of whether or not they met statistical criteria for confounding. Covariate data was assessed at each clinic visit (up to 4 visits) from 1984 to 1998. The risk associated with covariates was based on the covariate value at the clinic visit prior to the time of the CHD event. Clinic visits assessed all behavioral and clinical values for all covariates in this analysis. Missing data occurred when participants did not attend follow-up clinic visits however this was a very small number (7%) and covariate values from the baseline visit were used.

We also assessed the hazard ratio for CHD across arsenic exposure groups (TWA concentration, (20 to 30 μg/L, 30 to 45 μg/L and 45 to 88 μg/L relative to < 20 μg/L). The cutpoints for the exposure groups are based on arsenic concentrations in past research with significant associations with CHD (Moon et al, 2013; Chen et al. 2013).

We assessed whether hypertension might confound the association between inorganic arsenic exposure and CHD by reanalyzing the final model with hypertension as a dichotomous covariate. Lastly we completed secondary analyses to data collection methods and exposure estimates. The first was an agreement analysis on five percent of the interviewed participants (n=28) that compared residential address and year reported in the interview by the participant with the residential county clerk records. We compared residence and year for the year 1955- 1985 as reported by both sources. The next secondary analysis was completed in a limited cohort (n=462) to confirm any association found based on residential history using mean speciated urinary arsenic concentrations (As^3, As^5, MMA, and DMA). To complete this we compared urinary

arsenic concentrations between cases and non-cases as a secondary analysis. We used Statistical Analysis System 9.2 (PROC PHREG, SAS, version 9.2; SAS Institute, Inc, Cary, North Carolina) for the statistical analyses. We complied with all applicable requirements of national and international regulations including approval from Institutional Review Board and human participants provided written informed consent prior to participating in the study.

Results

This study included a cohort of 555 participants of which 96 were cases for a total of 6773 person years of follow-up (1984 through 1998 or CHD diagnosis). The subcohort had a median age of 57 years and was 53% white non-Hispanic (Table 1). Cases were ten years older than non-cases at the baseline visit, had higher percentages of non-Hispanic whites and males, and had higher LDL and triglyceride levels; however, cases and non-cases were similar with respect to family history of CHD, household income, smoking, BMI, physical activity, and consumption of alcohol and water (data not shown). The distributions of most CHD risk factors were not statistically different across arsenic exposure groups with exception of low income which had higher percent in the higher exposure groups (Table 2).

We estimated that a 15-μg/L increase in the TWA for residential inorganic arsenic water concentration was associated with a 36 percent higher risk for CHD (HR=1.36; 95% CI=1.06, 1.75 per 15 μg/L) (Univariate Model, Table 3). In a secondary analysis with TWA exposure categorized by four groups (Univariate Model, Table 3), we found a significant increase in the hazard ratio with increasing levels of arsenic exposure in a log rank test for trend (p=0.0007).

Estimates based on the final model also indicated a positive association with inorganic arsenic concentrations in drinking water (HR=1.41, 95% CI=1.09-1.81 per 15 μg/L) (Table 3, Full

Model). LDL and family history were significant risk factors and being female was a protective factor. The final adjusted model (Table 3, Final Model) showed that time-weighted average inorganic arsenic exposure maintained an association with increased risk for CHD (HR=1.38; 95% CI=1.09-1.78 per 15 μg/L) while adjusting for gender, family history of CHD and LDL levels. When inorganic arsenic exposure was categorized, the hazard ratios across exposure groups increased with increasing level of exposure ($p<0.0007$) while adjusting for gender, calculated LDL, and presence of a first degree family member with a CHD event (Table 4, Final Model). When hypertension was added to the final model, the association remained similar to the final model without hypertension (HR=1.36; 95% CI=1.06-1.74 per 15 μg/L) suggesting that the arsenic effect on CHD is likely operating through other mechanisms.

In the secondary analyses, we found that 73 percent of records matched between interview and county clerk records. We also found that cases had a statistically higher level of toxic urine arsenic species (As3, As5, MMA, and DMA) (μ=20.5 μg/g creatinine in non-cases verses μ=27.1 μg/g creatinine in cases; p=0.04).

Discussion

In this prospective study, we found that lifetime exposure to low levels of inorganic arsenic in drinking water (10 μg/L -100 μg/L) was associated with increased risk for coronary heart disease. We estimated that for every 15 μg/L increase in arsenic concentration in residential drinking water, the risk for CHD increased by 38 percent, and across increasing levels of exposure, risk increased in a dose-dependent fashion (trend p=0.0007) after adjusting for gender, family history of CHD, and serum LDL levels.

The wide spectrum of longitudinal clinical behavioral, and demographic data in SLVDS, plus a low rate of out-migration, along with variability in inorganic arsenic exposure in the San Luis Valley, renders this region and cohort particularly suitable for this research. Inorganic arsenic in groundwater in the SLV is natural, resulting primarily from weathering and erosion of rock formations (IDEQ 2002) and spatial variation is due to long-term patterns of rainfall and physio-chemical conditions (Abernathy et al. 2003; Hinwood et al. 2003).

We utilized a thorough residential and employment history, coupled with a comprehensive spatial prediction model of ground water concentrations of inorganic arsenic, to characterize a life course time-weighted average arsenic exposure at the individual level. The selection of residential arsenic concentration as the exposure metric was based on a correlation analysis with speciated arsenic concentrations in historically collected urine samples from this same cohort.

One plausible mechanism for arsenic cardiotoxicity is through the creation of oxygen radicals including lipid peroxidase, which can initiate endothelial cell proliferation, function, and apoptosis, a precursor to atherosclerosis (Chen et al. 2009; Hirano et al. 2003; Navas-Acien et al. 2005; Pi et al. 2002; Ratnaike 2003; Santra 2000; Waalkes et al. 2000). Studies in high arsenic areas of Asia have found increased levels of circulating reactive oxygen species such as hydrogen peroxide, hydroxyl radicals, and superoxide radicals (Yamanaka et al. 1990) and higher blood levels of lipid peroxidase (Pi et al. 2002) versus low exposure comparison groups. Other arsenic toxicity mechanisms that have been suggested include vascular smooth muscle cell proliferation and dysfunction (Bae et al. 2008), inhibited endothelial nitric oxide synthase activity (Kao et al. 2003; Lee et al. 2003), smooth muscle cell migration (Simeonova and Luster 2004) and enhanced platelet aggregation (Lee et al. 2002).

Past research has documented an association between hypertension and inorganic arsenic exposure (Chen CY et al. 1995, 2007; Rahmen et al. 1999); a systematic review of 11 studies (Abhyankar et al, 2012) also corroborated an association between arsenic exposure in drinking water and hypertension, even at low concentrations of arsenic. These findings suggest that arsenic may be related to CHD through a pathway that includes hypertension, a known risk factor for CHD; consequently, hypertension was not included as an independent risk factor in our models. We assessed hypertension as a potential confounder and found no change in the association between CHD and arsenic exposure. Also, although the study was adequately powered to investigate risk from arsenic exposure, there may be concern that well-known risk factors for CHD, including BMI and smoking, were not associated with CHD in this small cohort however other known CHD risk factors (family history of CHD, serum LDL levels, and gender) were significantly associated.

Recent research has suggested that intake of folate and selenium can influence arsenic metabolism and the association between cardiovascular disease (George et al. 2013); however, in this study, folate and selenium intake levels did not significantly contribute to the hazards model nor significantly change the association. This difference in finding could be due to variations in estimation of micronutrient intake in this study compared to others and therefore, should be a consideration to improve measurements for future research.

There exists the possibility of misclassification bias due to the exposure estimation in the use of exposure predication models and residential history reconstruction. Although our groundwater modeled predictions were correlated with arsenic concentrations measured in urine samples (ρ=0.63), misclassification cannot be ruled out. We also found in a limited cohort (that cases had a statistically higher level of toxic urine arsenic species suggesting that in a different metric of

exposure that an association between inorganic arsenic exposure and CHD also exists. Specific to the residential reconstruction, the primary method for data collection (interview) varied in response by case status (30% non-cases, 45% cases) although not by exposure status, which could induce misclassification bias. We believe that misclassification bias would be small given the low migration of this population (5 percent migrated to the SLV as children and only 10 participants (war veterans) lived outside of the SLV for more than 6 months (<3 years) through 1998) and the validation of clerk records and SLVDS database to complete residential history.

The exposure assessment does not include exposure resulting from ingesting contaminated food, inhalation of dust or soil, or tobacco products. In a comprehensive review of literature and analysis of arsenic, the Agency for Toxic Substances and Disease Registry (ATSDR) noted that in areas of the US where arsenic levels in drinking water are greater than 10μg/L, that ingestion of drinking water is the dominant source of inorganic arsenic exposure relative to the US diet and inhalation through air and therefore confirm our use of drinking water as the main source of exposure.

The exposure assessment remains limited by potential misclassification bias. Thirty-three percent of the subjects had a residential history created through records at the county clerk office because they were not available (e.g., deceased) for interview. However, the use of county clerk records were confirmed in participants who were not interviewed, where we found that data collected from county clerk records had strong agreement with self-reported residential history. For the subjects with imputed residence (n=18 subjects, 126 person years), we looked at city for the address prior to and after the period with missing residence and found an 89 percent agreement suggesting that many residents may move houses, but not necessarily city or out of the San Luis Valley. Self-reported estimates of lifetime residential, employment, and schooling locations and

duration, and number of cups of water consumed per day also are likely limited by inaccuracies leading to misclassification bias which could bias the findings.

Another limitation is that the arsenic exposure estimates were included in the proportional hazards model under the assumption of no error. Past research has incorporated bootstrap methods to incorporate an error term for the estimate in logistic regression models but to date has not been done in a proportional hazards model. A future step would be to develop the statistical methodology for incorporating the error term associated with the predicted arsenic exposure into the proportional hazards model.

A recent study, from a high arsenic area reported a hazard ratio for CHD of 1.22 (95% CI: 0.65, 2.32) at arsenic levels 12.1 to 62.0μg/L similar to levels found in the SLV while controlling for known CHD risk factors (Chen et al. 2011). Using a comprehensive exposure assessment, our study found consistent results at lower levels (1 to 100 μg/L) with a proportional hazards ratio of 1.75 for exposure levels from 30 to 45 μg/L relative to less than 20 μg/L. Our findings, plus those by Moon et al. 2013 who identified a similar association between incident cardiovascular disease and exposure to low to moderate arsenic levels with exposure defined through urine biomarkers, indicate that a dose-response relationship between arsenic and CHD exists at levels of arsenic that are not uncommon in many areas.

Inorganic arsenic exposure in drinking water has been identified as a cardiotoxic element at concentrations seen in drinking water supplies around the world which strengthens the importance of ensuring public water supplies meet the Environmental Protection Agency (EPA) maximum contaminant level (MCL) of 10 μg/L. Currently, there are many areas of the United States with levels above the EPA MCL including western states of Nevada, Colorado, and

Arizona; Midwest areas including Michigan, and Northeastern areas of New Hampshire, Maine and Connecticut (EPA 2011).

In conclusion, we observed an association between CHD risk and inorganic arsenic exposure in a chronic low-level arsenic area in southwestern US. Because arsenic in drinking water remains a common exposure in the United States, the risk of CHD should provide motivation to public health officials to bring drinking water levels into compliance (below 10 μg/L) and to conduct further research to elucidate the role of arsenic in the pathobiology of CHD.

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Table 1. Baseline demographic, clinical, and behavioral characteristics of study participants with and without incident CHD during follow-up (n = 555).

Variable	**Total Subcohort N=555**
Arsenic Exposure TWA	
1-20 µg/L-yr	428 (77%)
20-30 µg/L-yr	86 (15%)
30-45 µg/L-yr	33 (6%)
45-88 µg/L-yr	8 (1%)
Age (baseline) (median, IQR)	57 (46, 64)
Ethnicity	
White non-Hispanic	296 (53%)
Hispanic	259 (47%)
Gender	
Male	267 (46%)
Female	288 (54%)
Income	
Low	304 (47%)
High	251 (53%)
First Degree Family History of CHD	
No	439 (81%)
Yes	116 (19%)
BMI (median, IQR) (n=1 missing)	26.0 (23.7, 29.4)
Diabetic (diagnosed at baseline visit)	
No	546 (98%)
Yes	9 (2%)
Current Smoker	
Yes	262 (48%)
No	293 (52%)
Alcohol	
≤168 grams/week	528 (95%)
>168 grams/week	27 (5%)
Water Consumption	
<5cups/day	225 (39%)
≥5 cups/day	330 (61%)
Physical Activity	
Sedentary	367 (69%)
Active	188 (31%)
Serum LDL (mg/dl) (median, IQR) (n=10 missing)	134 (108, 163)
Serum HDL(mg/dl) (median, IQR) (n=4 missing)	46 (38, 56)
Serum Triglycerides (mg/dl) (median, IQR) (n=4 missing)	145 (102, 197)
Folate (mg) (median, IQR) (dietary assessment estimate)	257 (183, 137)
Selenium (mg) (medium, IQR) (dietary assessment estimate)	98 (89, 48)

Table 2. Baseline demographic, clinical, and behavioral risk factors for CHD across time-weighted average arsenic exposure groups (n=555).

Variable	1–20 µg/L-yr N=428	20–30 µg/L-yr N=86	30–45 µg/L-yr N=33	45-88 µg/L-yr N=8
Age (median, IQR)	67 (59, 73)	68 (62, 73)	69 (63, 73)	69 (66, 75)
Ethnicity				
White non-Hispanic	218 (51%)	58 (67%)	15 (5%)	5 (63%)
Hispanic	210 (49%)	28 (33%)	18 (54%)	3 (37%)
Low Income (<$20, 000)*	257 (60%)	25 (29%)	18 (55%)	4 (50%)
Gender				
Male	208 (49%)	40 (47%)	15 (45%)	4 (50%)
Female	223 (52%)	46 (53%)	17 (53%)	2 (25%)
Family History	94 (22%)	17 (20%)	3 (9%)	2 (25%)
BMI (median, IQR)	27.11 (23.51, 29.52)	26.43 (23.42, 28.40)	27.20 (25.84, 29.61)	25.31 (21.02, 28.22)
Diabetic (diagnosed at baseline visit)	8 (2%)	0	1 (4%)	0
Current Smoker	208 (49%)	40 (47%)	11 (33%)	3 (37%)
Alcohol >168 grams/week	18 (4%)	7 (9%)	2 (6%)	0
Water Consumption ≥5 cups/day	264 (62%)	39 (45%)	22 (66%)	5 (63%)
Sedentary Physical Activity	291 (68%)	55 (64%)	18 (64%)	3 (37%)
Serum LDL (mg/L) (median, IQR)	257 (104, 159)	126 (102, 154)	125 (107, 151)	132 (101, 151)
Serum HDL (mg/L) (median, IQR)	88 (35, 55)	46 (38, 54)	43 (36, 51)	43 (34, 56)
Serum Triglycerides (mg/L) (median, IQR)	294 (98, 208)	162 (138, 227)	163 (138, 227)	124 (87, 139)
Folate (mg) (median, IQR)	234 (158, 157)	277 (235, 217)	320 (253, 304)	232 (176, 66)
Selenium (mg) (median, IQR)	89 (83, 52)	119 (106, 71)	114 (93, 58)	98 (90, 32)

IQR: inter-quartile range.

*Statistically different in Chi-sq across groups $p<0.05$.

Table 3. Cox proportional hazards modeling results for the primary analysis of the association between CHD and time-weighted average (TWA) inorganic arsenic exposure as a continuous variable.

Variable	Univariate Model HR(95% CI)	Full Model HR(95% CI)	Final Model HR(95%CI)
Arsenic Exposure TWA (15 µg/L)[a]	1.36 (1.11, 1.82)	1.41 (1.09, 1.81)	1.38 (1.09, 1.78)
Female Gender		0.38 (0.23, 0.64)	0.35 (0.19, 0.53)
Hispanic Ethnicity		1.12 (0.70, 1.88)	
Primary Family Member Diagnosed with CHD		1.68 (0.98, 2.89)	1.75 (1.07, 2.88)
Low Income		1.17 (0.67, 2.06)	
Diabetic (diagnosed at baseline visit)		1.18 (0.15, 9.52)	
BMI (per 5.5 kg/m^2)[a]		0.81 (0.54, 1.20)	
Sedentary Physical Activity		1.11 (0.69, 1.79)	
Current Smoker		1.02 (0.63, 1.65)	
High Alcohol Consumption		1.76 (0.70, 4.41)	
Low Density Cholesterol (53 µg/dl)[a]		1.47 (1.05, 2.07)	1.40 (1.04, 1.88)
High Density Cholesterol (17 µg/dl)[a]		0.64 (0.43, 1.01)	
Triglycerides (90 µg/dl)[a]		0.94 (0.67, 1.34)	
Folate (57 mcg)[a]		1.00 (0.99, 1.00)	
Selenium (185 mcg)[a]		0.99 (0.99, 1.00)	

Univariate Model: proportional hazards model with TWA arsenic exposure (main risk factor) as independent variable.

Full Model: proportional hazards model with TWA arsenic exposure (main risk factor) and all listed variables as time dependent independent variables.

Final Model: proportional hazards model with TWA arsenic exposure (main risk factor) and statistically significant covariates (independent variables).

Time dependent covariates are: BMI, Physical activity, Smoking status, Alcohol consumption, serum lipid levels, and micronutrient intake.

[a]Inter-quartile range of subcohort at baseline.

Table 4. Cox proportional hazards modeling results for the secondary analysis of the association between CHD and categorical time-weighted average (TWA) inorganic arsenic exposure.

Variable	Univariate Model HR(95%CI)	Full Model HR(95%CI)	Final Model HR(95%CI)
Arsenic Exposure TWA			
1-20 µg/L-yr[a]	1.0	1.0	1.0
20-30 µg/L-yr[b]	1.24 (0.70, 2.31)	1.25 (0.60, 2.61)	1.23 (0.56, 2.18)
30-45 µg/L-yr[c]	2.14 (1.22, 3.98)	2.08 (1.11, 3.92)	2.18 (1.23, 4.02)
45-88 µg/L-yr[d]	3.12 (1.11, 9.02)	3.34 (1.15, 9.30)	3.10 (1.10, 9.11)
Female Gender		0.41 (0.24, 0.69)	0.35 (0.19, 0.48)
Hispanic Ethnicity		1.01 (0.62, 1.70)	
Primary Family Member Diagnosed with CHD		1.55 (0.88, 2.70)	1.63 (0.94, 2.82)
Low Income		1.21 (0.71, 2.20)	
Diabetic (diagnosed at baseline visit)		2.05 (0.51, 9.21)	
BMI (per 5.5 kg/m^2)		0.85 (0.59, 1.22)	
Sedentary Physical Activity		1.12 (0.65, 1.80)	
Current Smoker		1.03 (0.63, 1.68)	
High Alcohol Consumption		1.70 (0.72, 4.10)	
Low Density Cholesterol (53 µg/dl)[e]		1.46 (1.03, 2.08)	1.40 (1.02, 1.99)
Triglycerides (90 µg/dl)[e*]		0.94 (0.65, 1.34)	
High Density Cholesterol (17 µg/dl)[e]		0.67 (0.43, 1.02)	
Folate (57 mcg)[e]		1.00 (0.99, 1.01)	
Selenium (185 mcg)[e]		1.00 (0.99, 1.03)	

Univariate Model: proportional hazards model with TWA arsenic exposure (main risk factor) as independent variable.

Full Model: proportional hazards model with TWA arsenic exposure (main risk factor) and all listed variables as time dependent independent variables.

Final Model: proportional hazards model with TWA arsenic exposure (main risk factor) and statistically significant covariates (independent variables).

Time dependent covariates are: BMI, Physical activity, Smoking status, Alcohol consumption, serum lipid levels, and micronutrient intake.

[a]Person years of follow up = 4806. [b]Person years of follow up = 1335. [c]Person years of follow up = 534. [d]Person years of follow up = 98. [e]Inter-quartile range of subcohort at baseline.